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QTL fine mapping and identification of candidate genes for growth-related traits in bighead carp (*Hypophthalmichehys nobilis*)

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ABSTRACT

Bighead carp (*Hypophthalmichthys nobilis*) is a popular Asian aquaculture species, but its genetic improvement is still in infancy. Marker-assisted selection (MAS) can improve the selection efficiency of breeding programs in fish. In this study, we constructed a genetic linkage map in a F1 family of bighead carp using microsatellite markers. A total of 905 microsatellites were assigned onto 24 linkage groups (LGs) of a consensus map, which spanned 1631.7 cM of bighead carp genome with an average interval of 1.8 cM. Comparative genomics revealed a high level of genomic synteny between bighead carp and zebrafish. QTL mapping for growth traits was performed based on this linkage map, and three significant and 8 suggestive QTL associated with four growth traits (body length, body height, head length, body weight) were detected on LG9 and LG17, with 18.6–25.5% of phenotypic variance explained. Three candidate genes for growth were identified in or near the QTL intervals, and a SNP in one of the three genes, *TP53BP2*, was significantly associated with growth traits in different populations of bighead carp. These results of the high-density SSR genetic map and genome scan for QTL provide a basis for MAS and breeding programs for growth in bighead carp.

1. Introduction

Growth is an important trait of interest in economic aquaculture animals, and it is controlled by multiple genes (quantitative trait loci, QTL) and environmental factors (Mackay, 2001; Massault et al., 2008). Faster growth rate is one of the main goals of many breeding programs for fish because it shortens rearing time and increases benefits for aquaculture industry. Traditional methods of genetic improvement have relied mainly on family and individual selection based on phenotype and pedigree information (Hulata, 2001). With the development of molecular biotechnology, marker-assisted selection (MAS) has been developed and applied in many fish genetic breeding programs. Besides its accuracy and efficiency, MAS is less labor and time consuming compared with traditional breeding methods (Sonesson and Meuwissen, 2009). The first step of MAS is to identify available genetic markers or genes associated with target traits. QTL mapping based on the phenotypic and genotypic data of mapping family provides a powerful method of founding these relationships on a genome-wide scale (Lande and Thompson, 1990; Mackay et al., 2009).

Genetic linkage map is an essential tool for QTL mapping and other genetic and genomic researches, such as comparative genomics, positional gene cloning, gene-centromere mapping and genome assembly (Lynch and Walsh, 1998; Yue, 2014). Compared with other popular molecular markers, microsatellite, or simple sequence repeat (SSR) marker is one of the best options for linkage map construction because of its many merits, such as abundance in genome, uniformly distribution, high polymorphism, co-dominant inheritance, long flanking sequences, ease of detection by PCR, and easy accession by other laboratories via published primer sequences (Liu and Cordes, 2004). Several high-density genetic linkage maps have been constructed based on microsatellites for aquaculture species, such as Asian seabass (Wang et al., 2011a), Japanese flounder (Song et al., 2012b), half-smooth tongue sole (Song et al., 2012a). QTL for disease resistance, salinity and temperature tolerance, sex determination and growth traits have been identified in various fish species based on microsatellite-based genetic maps (Laghari et al., 2014; Yue, 2014). Given the importance of production in aquaculture, QTL for growth traits have been investigated in over 20 aquaculture species, such as rainbow trout (Wringe et al., 2010), Atlantic salmon (Gutierrez et al., 2012), Arctic charr (Kuttner et al., 2011), European sea bass (Louro et al., 2016), large yellow croaker (Xiao et al., 2015), Japanese flounder (Song et al., 2012a), small abalone (Ren et al., 2016), triangle pearl mussel (Bai et al., 2015) and Chinese mitten crab (Qiu et al., 2016). A lot of candidate genes and molecular markers have been identified based on the results of QTL mapping and provide useful tools for MAS programs to improve the efficiency and precision of fish breeding (Liu et al., 2014; Wang et al., 2015b; Xia et al., 2013).

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Bighead carp is one of the most important commercial aquaculture fish in China, and it is not only consumed as edible fish but also used to control water quality in many other countries. The global aquaculture production of this species was 3.25 million tons and its economic value reached 4.19 billion dollars in 2014 (FAO). However, natural populations of bighead carp have seriously declined during recent decades due to ongoing human activities such as overfishing and pollution. Worse still, unscientific artificial releasing and frequent flooding may lead to a mixture of natural and cultured individuals, which has resulted in in-breeding depression and reduction of growth performance (Liu et al., 1997; Zhu et al., 2014). Furthermore, the traditional selective breeding program is inefficient in bighead carp because of its relatively long generation interval (about 4–5 years). Hence, it's necessary to deploy MAS program and accelerate the breeding program for bighead carp. Although some genetic and genomic resources have been developed in bighead carp, such as microsatellite markers (Feng et al., 2014; Guo et al., 2013a; Zhu et al., 2013a), the first and second generation genetic linkage map (Liao et al., 2007; Zhu et al., 2014), microsatellite-centromere mapping (Zhu et al., 2013b) and comparative genomics (Zhu et al., 2015), the genetic architecture of economic traits remains unknown.

The objectives of this study include: (i) construction of a high-density SSR-based linkage map; (ii) QTL fine mapping for growth traits; (iii) identification of candidate genes associated with growth traits. The high-density linkage map will build a foundation for fine QTL mapping and detection of candidate genes that may affect growth traits in bighead carp, a large domestic cyprinid fish with significance to China's and world aquaculture industry.

2. Materials and methods

2.1. Mapping family

Adult male and female bighead carp were collected from different sections of the Yangtze River and raised in muddy ponds at the Zhangdu Lake Fish Farm (Wuhan, China). In May 2011, genetic distances among 50 matured bighead carp were evaluated by using 10 polymorphic microsatellite markers. 5 dams and 5 sires with relatively higher genetic distance (0.21–0.30) were selected to produce a multiple-family population by mass crossing in artificial propagation. About 2000 fry were raised in an about 0.6 ha (60 hm^2) muddy pond. In December 2011, 840 progenies were randomly collected and five growth-related phenotypic traits were measured, i.e. body length (BL), body height (BH), head length (HL) and body weight (BW). Fin tissues were sampled from both parents and progenies and preserved in anhydrous ethanol at 4 °C. Genomic DNA was extracted from fin tissues following a standard phenol-chloroform protocol (Sambrook and Russell, 2001). All parents and progenies were genotyped by 8 highly polymorphic microsatellites to verify parent-offspring relationships. Parentage assignment for the mixed families was estimated using the likelihood-based approach with the program Cervus 3.0 (Kalinowski et al., 2007), and each progeny was correctly assigned to a single parental pair (100% success). Finally, a F1 full-sib family with 90 progenies exhibiting high variation in growth traits was selected as the mapping population for genetic map and QTL in this study. The correlation analysis among four growth traits and normal distribution test for four growth traits were implemented using a software package SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

To verify our QTL results in different population and different environment, in May 2012, 6 dams and 6 sires were randomly selected to produce multiply-family population by mass crossing in artificial propagation. A total of 36 families were produced and then raised in the same muddy pond. In December 2012, 414 progenies were randomly collected and four growth-related phenotypic traits (BL, BH, HL and BW) were measured. Two extreme groups (large size group: 41 fishes, and small size group: 41 fishes) for growth traits were selected from the verification population and used for association analysis.

2.2. SSR markers and genotyping

To test the feasibility of PCR application and genetic segregation in the mapping panel, a total of 4325 microsatellite markers were used in the initial screening. These microsatellite markers were recruited from four sources: (1) 800 bighead carp markers (Arsd) and 1912 silver carp markers (Hysd) were developed from genome sequencing (Guo et al., 2013b; Zhu et al., 2014), (2) 406 markers (HysdE) were developed through silver carp transcriptome sequences (Feng et al., 2014), (3) 775 markers were developed from three microsatellite-enriched genomic libraries, with 559 from bighead carp assigned with the prefixes 'ArGA' and 'ArGT' (Guo et al., 2013b; Zhu et al., 2014), 216 from silver carp assigned with the prefixes 'HyGT' (Guo et al., 2013b; Zhu et al., 2014), (4) 432 microsatellites which were developed and used in silver carp genetic map with the prefixes 'Hym' (Zhang et al., 2010) were also applied in this study.

All microsatellite markers mentioned above were applied to initial polymorphism screening by genotyping two parents and four progenies. Those segregated markers were subsequently genotyped in the whole mapping population to generate genotype data for the construction of a genetic linkage map in bighead carp. PCR reactions were carried out in a ABI Veriti 96 well thermal cycler with a total volume of 12.5 μl, containing 30–50 ng of template DNA, 1.25 μl of 10× reaction buffer, 1 U of Taq polymerase (TaKaRa, Japan), 0.5 μl of dNTP (2.5 mmol/l), 0.5 μl of forward and reverse primer mixture (2.5 μmol/l each) and water to the final volume. The PCR programs were as follows: 94 °C for 4 min, 35 cycles at 94 °C for 35 s, optimal annealing temperature (Additional File 1) for 35 s, 72 °C for 40 s, and a final extension of 72 °C for 10 min. The PCR products were separated on 8% polyacrylamide gels (PAGE) and visualized by the JS-780 gel imaging system (Peiqing, China) after staining by ethidium bromide.

2.3. Map construction and estimation of genome size

The linkage analysis was implemented by JoinMap 4.0 program (Van Ooijen, 2006) using the CP (cross pollination) population type, and five CP separation types (nn × np, lm × ll, hk × hk, ef × eg, ab × cd) were presented in two parents and 90 progenies. Markers with >10% missing data were eliminated from further analysis. Chi-square tests were performed to determine the fitness of marker segregation data to the expected 1:1 ratio. Distorted markers were also used for linkage analysis, and they were indicated with asterisks on LGs. The sex-averaged linkage map was constructed using the function of "Create Population Nodes" in the JoinMap 4.0 program. A logarithm of odd (LOD) threshold of 5.0 was set for clustering markers into linkage groups (LGs). Graphical representations of linkage groups were created using the software MapChart 2.2 (Voorrips, 2002). LGs were named according to the chromosome assignments corresponding to homologous groups of the previous linkage map for bighead carp (Zhu et al., 2014).

The estimated genome lengths for consensus map were calculated based on two different approaches: (1) Ge1 was calculated as the method described (Fishman et al., 2001), (2) Ge2 was calculated by a different method (Chakravarti et al., 1991). The estimated genome lengths (Ge) are the averages of the lengths calculated by these two methods.

2.4. Comparative genomics with zebrafish genome

Flanking sequences of the SSR markers assigned on the genetic map of bighead carp were used to search against *Danio rerio* genome (GRCz10) using NCBI-BLAST-2.2.31+ (BLASTN) with the expect value (e-value) less than $1e^{-10}$. If a single marker sequence was aligned to the *D. rerio* genome with multiple positions, only the alignment with the lowest e-value was reserved. The genomic synteny between bighead carp and zebrafish was visualized using the software Circos v0.67 (Krzywinski et al., 2009).

2.5. QTL analysis

QTL mapping for growth traits was performed with MapQTL version 6.0 (Van Ooijen and Kyazma, 2009). Multiple QTL Mapping (MQM) method was employed to detect any significant associations between markers and growth-related traits. LOD statistics were calculated at an interval distance of 1 cM. The genome-wide (significance level) and linkage group-wide (suggestive level) LOD threshold were estimated using the permutation test (10,000 replicates) with a confidence interval of 95%. Graphic illustrations of the QTL profiles were generated using the program MapChart 2.2 (Voorrips, 2002).

2.6. Identification and molecular cloning of candidate genes

Potential candidate genes within each QTL region were identified through comparative genomics. We performed a sequence similarity search for flanking sequences of those QTL-linked SSR markers against the whole genome sequences of zebrafish (GRCz10) and bighead carp (Shunping He et al. unpublished data). Only annotated genes located closest to the peak of corresponding QTL region were regarded as candidate genes. cDNA sequences of the selected candidate genes were obtained from total RNA by homology cloning and 3′- and 5′-RACE methods.

2.7. Association analysis

In order to investigate associations between growth traits and genotypes of the candidate genes, two extreme groups ($N = 41$/group, large and small 10% individuals) for growth traits were selected from the verification population. The sequences of exons, 3′ and 5′UTR regions of a given candidate gene were screened in 22 parents of mapping population and verification population and two extreme groups of verification population to identify gene polymorphisms. The associations between genotypes of polymorphic sites and growth traits were analyzed using the General Linear Model (GLM) of SPSS 19.0 with same parameters and significance level as we applied before (Liu et al., 2012; Sun et al., 2012).

3. Results

3.1. Growth traits

In the *F*1 mapping family, the four growth traits, BL, BH, HL and BW, all fitted normal distributions, with average values of 25.084 ± 1.374 cm, 7.577 ± 0.406 cm, 9.343 ± 0.461 cm, and 0.356 ± 0.052 kg, respectively. The results of correlation analysis showed that these growth traits are significantly correlated ($P < 0.01$) with each other (Table 1). Among them, the highest correlation coefficient value ($r = 0.967$) was observed between BL and BW, followed by that between BH and BW ($r = 0.920$), while the weakest correlation was found between HL and BH ($r = 0.774$).

3.2. Consensus linkage map

A total of 928 markers including 812 SSRs and 116 EST-SSRs were segregated in the mapping family and used for map construction. A total of 68 markers were distorted, and the distorted markers were also used for linkage analysis. In the construction of consensus map with JoinMap 4.0, linkage analyses were applied with an LOD threshold of 5.0. A total of 905 markers were assigned into 24 linkage groups (LGs), which were in accordance with the haploid chromosome numbers of the bighead carp ($2N = 48$). The information of SSR markers and the sex-averaged linkage map were presented in Additional File 1. The total length of the consensus map was 1631.7 cM with an average inter-marker distance of 1.8 cM (Table 2 and Fig. 1). The length of LGs ranged from 43.4 cM (LG24) to 107.7 cM (LG15) with an average of 68.0 cM. The number of markers in each LG varied from 23 to 70, with an average of 37.8. Based on two different approaches, the estimated genome lengths of the bighead carp genome were 1718.1 cM (Ge1) and 1727.8 cM (Ge2), respectively, with an average of 1722.9 cM (Ge). And the genome coverage of this consensus map was 94.7%. Among the 905 SSRs, 67 (7.4%) were not in accordance with the Mendelian expectations. These distorted markers were not evenly distributed in the map, instead, they were mainly clustered in LG4, LG7, LG10, LG19 and LG21 (Table 2; Fig. 1).

Table 1
Pearson's correlation between growth quantitative traits in F1 progeny($n = 90$).

	BL	BH	HL	BW
BL				
BH	0.838**			
HL	0.861**	0.774**		
BW	0.967**	0.920**	0.867**	

BL body length, *BH* body height, *HL* head length, *BW* body weight.
** $P < 0.01$.

3.3. Comparative genome analysis

A high level of syntenic relationship was observed between bighead carp LGs and zebrafish chromosomes. A total of 715 markers were uniquely aligned to zebrafish genome (Fig. 2a), with 578 (80.8%) markers located into 25 syntenic boxes (Fig. 2b). The linkage group, LG9 of bighead carp was homologous with two chromosomes (chr 10 and chr 22) of zebrafish genome, and other LGs showed a 1:1 syntenic relationship with zebrafish chromosomes (Fig. 2a, Fig. 2b).

Table 2
Properties of the consensus linkage map of bighead carp.

Linkage group	No. of markers	Length (cM)	cM/marker	No. of SDL	Gm1	Gm2	G
LG1	52	82.2	1.6	1	85.8	85.4	85.6
LG2	36	58.8	1.6	1	62.4	62.2	62.3
LG3	70	75.3	1.1	0	78.9	77.5	78.2
LG4	27	61.8	2.3	9	65.4	66.6	66.0
LG5	24	67.0	2.8	2	70.6	72.9	71.7
LG6	48	59.6	1.2	0	63.2	62.1	62.6
LG7	36	56.9	1.6	10	60.5	60.2	60.4
LG8	42	78.8	1.9	2	82.4	82.7	82.6
LG9	36	75.3	2.1	0	78.9	79.6	79.3
LG10	43	70.1	1.6	5	73.7	73.5	73.6
LG11	28	62.8	2.2	2	66.4	67.4	66.9
LG12	23	63.1	2.7	4	66.7	68.8	67.7
LG13	57	63.0	1.1	1	66.6	65.3	65.9
LG14	39	64.8	1.7	4	68.4	68.2	68.3
LG15	28	107.7	3.8	1	111.3	115.6	113.4
LG16	55	102.3	1.9	0	105.9	106.1	106.0
LG17	45	58.5	1.3	0	62.1	61.1	61.6
LG18	29	53.7	1.9	1	57.3	57.6	57.5
LG19	24	63.6	2.7	14	67.2	69.1	68.2
LG20	28	48.4	1.7	0	52.0	51.9	52.0
LG21	42	73.3	1.7	6	76.9	76.9	76.9
LG22	39	68.8	1.8	0	72.4	72.4	72.4
LG23	25	72.4	2.9	1	76.0	78.4	77.2
LG24	32	43.4	1.4	3	47.0	46.2	46.6
Total	905	1631.7		67			
Average	37.8	68.0	1.8				
Expected genome length (cM)					1718.1	1727.8	1722.9
Genome coverage (%)					95.0%	94.4%	94.7%

Fig. 1. Genetic linkage map for bighead carp based on microsatellite markers. The genetic distances in Kosambi centimorgans are listed on the left of each LG, and markers are listed on the right. Distorted markers are marked with "*". The locations of quantitative trait loci (QTL) affecting growth-related traits are shown in the linkage groups 9 and 17.

3.4. QTL mapping for growth traits and potential candidate genes

Totally, three significant and 11 suggestive QTL associated with four growth traits were detected on LG9 and LG17, accounting for 18.6–25.5% of the phenotypic variance explained (PVE) (Table 3; Figs. 1 and 3). On LG9, one QTL interval was situated from 30.64 to 33.25 cM, and was considered as the major QTL in this study. In this interval, three genome-wide significant QTL (qBL9-a, qBH9-a, qBW9-a) related to BL, BH, BW. Those three QTL LOD scores ranged from 4.44 to 4.78, and explained 23.9% to 25.5% of the PVE. The nearest SSR to the peak of this significant QTL region was Arsd215.

On LG 17, two main QTL regions, 40.10–40.83 cM and 46.87–48.00 cM, were detected for four growth traits (Table 3; Figs. 1 and 3). In the former region, four group-wide suggestive QTL (qBL17-a, qHL17-a, qBH17-a, qBW17-a) were identified with the LOD scores of 3.63, 3.67, 3.35 and 3.74, and PVE of 20.0, 20.2, 18.6, and 20.5%,

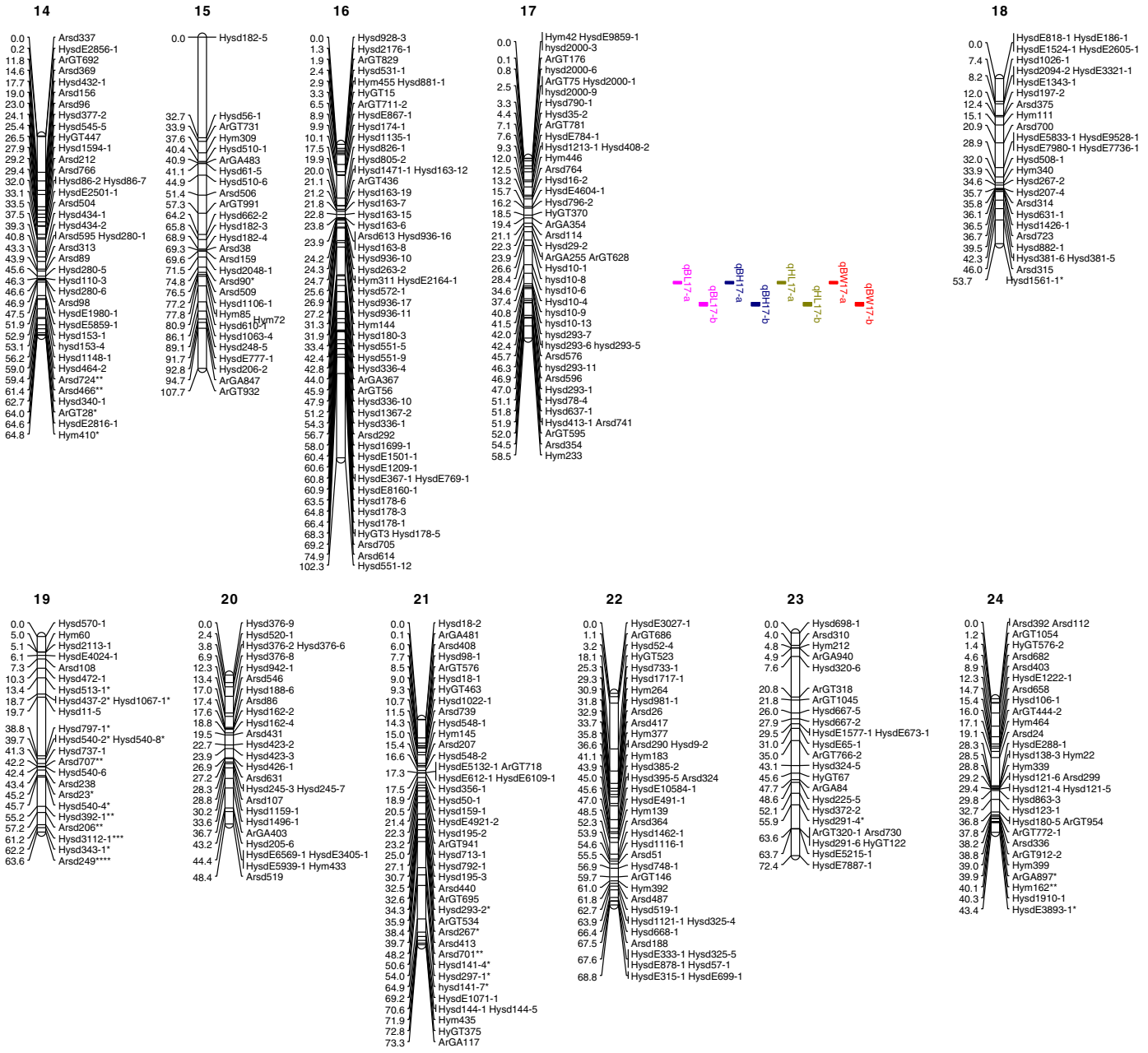

Fig. 1 (*continued*).

respectively. A single SSR marker (Hysd10-9) located in this QTL region. In the later QTL region, four group-wide suggestive QTL (qBL17-b, qHL17-b, qBH17-b, qBW17-b) were detected, with QTL scores ranging from 3.61 to 3.82, and PVE of 19.9%–20.9%. The nearest SSR to the peak of this QTL region was Hysd293-1.

Three markers (Arsd215, Hysd10-9 and Hysd293-1) located closest to the peak of QTL regions were BLASTn aligned against whole genome sequences of zebrafish and bighead carp to detected potential functional genes for growth. Results showed that Arsd215 on LG9 corresponded to *TP53BP2* (*tumor protein p53 binding protein 2*) in bighead cap genome and zebrafish genome, and Hysd10-9 and Hysd293-1 on LG17 corresponded to *Tle3a* (transducin-like enhancer of split 3a) and *Ankrd34c* (ankyrin repeat domain 34C), respectively.

3.5. SNP identification and association analysis

SNPs from the candidate gene *TP53BP2* were tested for their possible associations with growth in the verification population. The polymorphisms of the *TP53BP2* were detected by direct sequencing of PCR products amplified by primer pairs spanning the exons. Six polymorphic sites were identified in 13 exons of TP53BP2, while five of these loci (c454C>G and c617G>T in exon 5, c1301G>C in exon 10, c1361T>C and c1439G>A in exon 11) showed severe segregation distortion as different genotypes were detected in only two of the 22 parents. Moreover, all of these five loci were monomorphic in the 12 parents of the verification population. Thus, these five loci were not used for further analysis. The SNP mutation TP53BP2-c813C>T in exon 7 was in the HWE, and then genotyped in verification population. As a result, the SNP

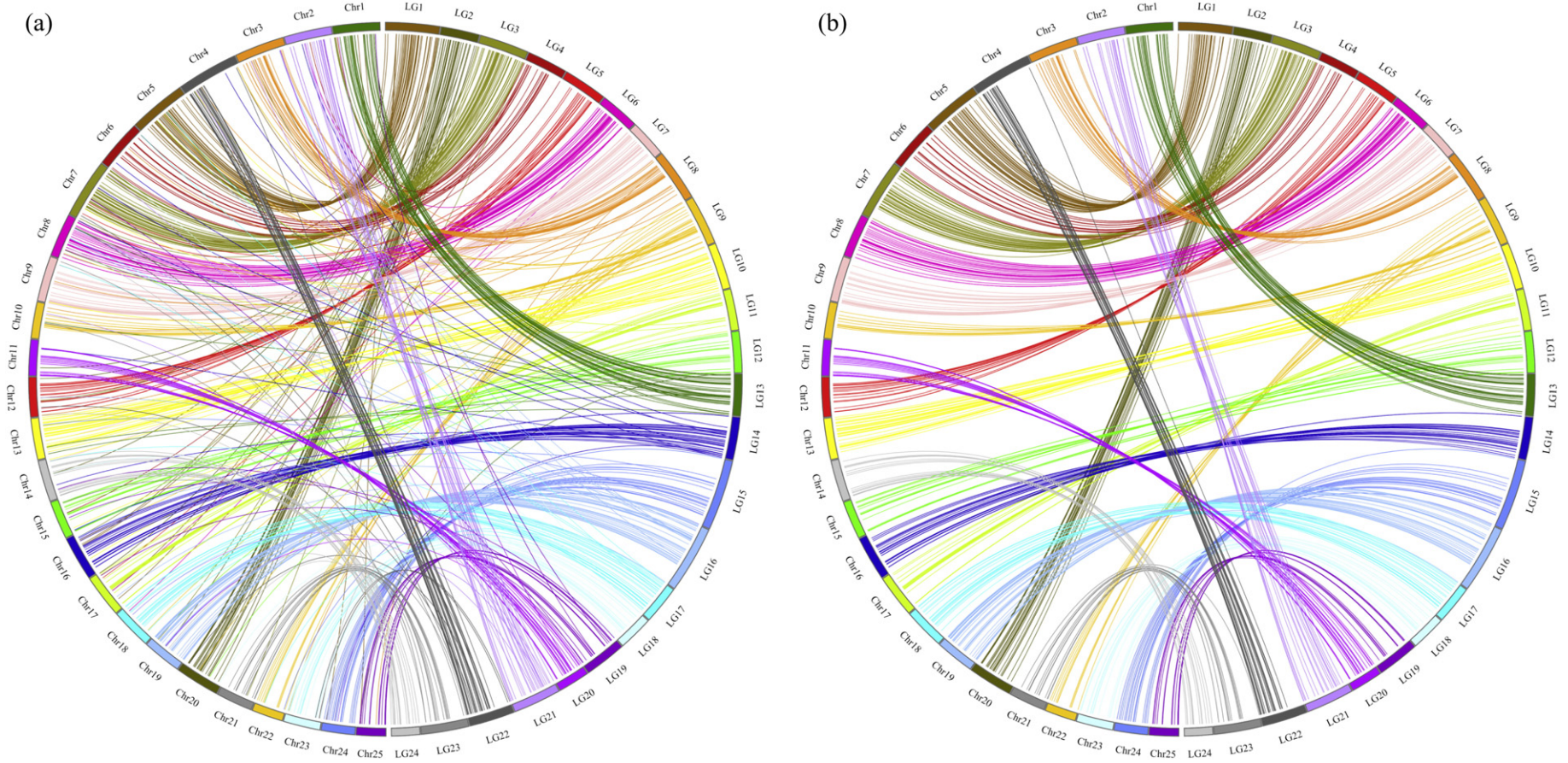

Fig. 2. Circos diagram representing syntenic relationships between bighead carp and zebrafish (a). Only markers on each linkage group of bighead carp that were mapped to a single chromosome of zabrafish were shown in (b).

Table 3
Location of QTL and magnitude of QTL effects related growth traits.

Trait	QTL name	QTL region(cM)	Markers	LG	Position(cM)	LOD	LOD Threshold		PVE(%)
							Genome	Group	
BL	qBL9-a	30.64–33.25	Arsd215	9	32.03	4.78	4.4	3.7	25.5
	qBL17-a	40.10–40.83	Hysd10-9	17	40.83	3.63	4.4	3.6	20
	qBL17-b	46.87–48.00	Hysd293-1	17	48.00	3.71	4.4	3.6	20.4
BH	qBH9-a	30.64–33.25	Arsd215	9	32.03	4.55	4.4	3.3	24.4
	qBH17-a	40.10–40.83	Hysd10-9	17	40.83	3.67	4.4	3.3	20.2
	qBH17-b	46.87–48.00	Hysd293-1	17	48.00	3.61	4.4	3.3	19.9
HL	qHL17-a	40.10–40.83	Hysd10-9	17	40.83	3.35	4.3	3.3	18.6
	qHL17-b	46.87–48.00	Hysd293-1	17	48.00	3.79	4.3	3.3	20.8
BW	qBW9-a	30.64–33.25	Arsd215	9	32.03	4.44	4.4	3.4	23.9
	qBW17-a	40.10–40.83	Hysd10-9	17	40.83	3.74	4.4	3.3	20.5
	qBW17-b	46.87–48.00	Hysd293-1	17	48.00	3.82	4.4	3.3	20.9

TP53BP2-c813C>T in exon 7 was significantly associated with BL, BH, HL and BW, and individuals with genotype CC had much higher values of growth traits than those with genotype TT ($P < 0.05$) (Table 4; Fig. 4).

4. Discussion

4.1. Genetic linkage map

In recent years, a number of high-density genetic linkage maps have been constructed in aquaculture species based on microsatellites, such as Asian seabass (790 SSRs) (Wang et al., 2011a), Atlantic salmon (1179 SSRs) (Danzmann et al., 2008), Japanese flounder (1487 SSRs) (Song et al., 2012b), half-smooth tongue sole (1007 SSRs) (Song et al., 2012a), silver carp (703 SSRs) (Guo et al., 2013b) and kelp grouper (714 SSRs) (Kessuwan et al., 2015). In this study, a new genetic linkage map of bighead carp was constructed based on 905 SSRs, with an average marker interval of 1.8 cM which was slightly lower than Japanese flounder (1.35 cM) and half-smooth tongue sole (1.67 cM), but higher than other three maps mentioned above (2.2 cM–4.1 cM). This present genetic map also significantly improved our previous linkage map (Zhu et al., 2014) by both resolution and accuracy, which would be useful for QTL mapping and identification of potential genes for growth in bighead carp. Genetic linkage maps with more markers may have a larger map size, but other factors such as chromosome interferences may also affect the size of a genetic linkage map (Li and He, 2014; Sun and Liang, 2004). The total length of this new linkage map shorter than the previous map may be because different chromosome interferences derived from interspecific hybridization (Zhu et al., 2014) and intraspecific cross (this study).

Distorted markers at specific LG regions may link to deleterious alleles or lethal genes (Launey and Hedgecock, 2001; Young et al., 1998). In this study, we used all distorted SSRs in the map construction,

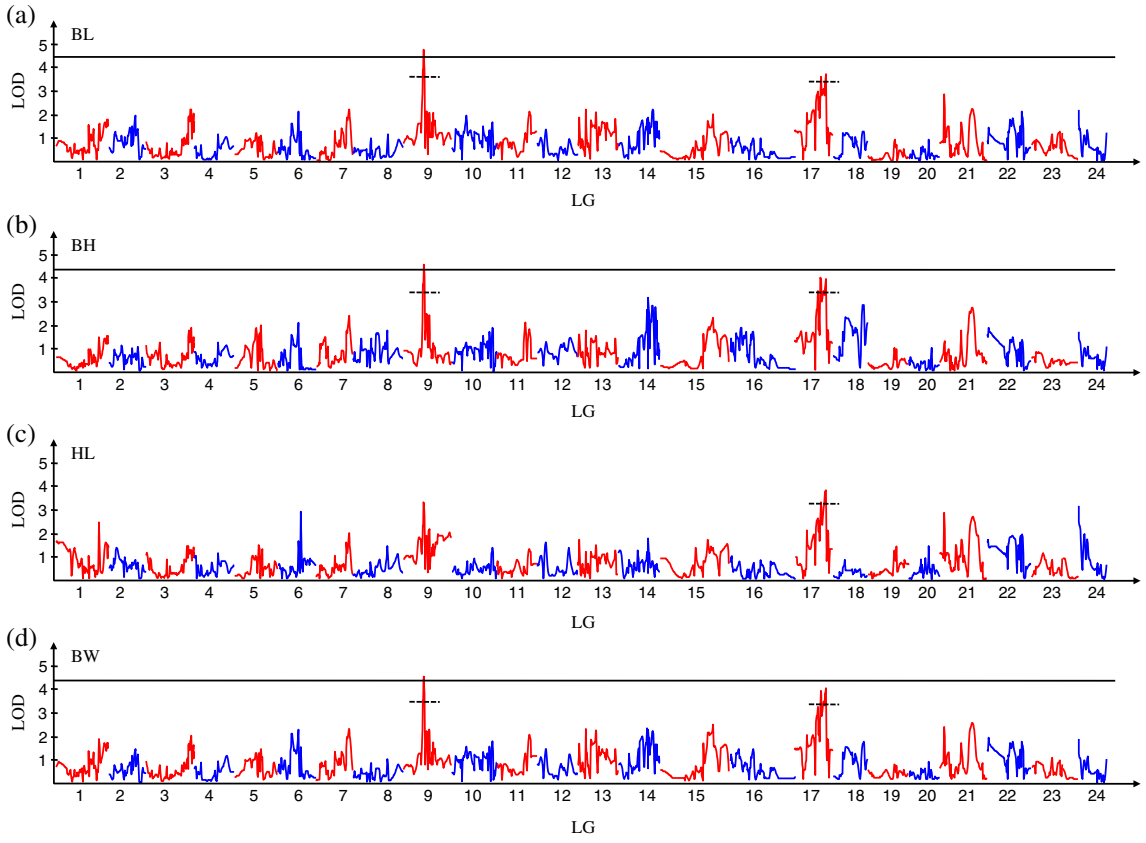

Fig. 3. A genome scan of LOD profiles for body length, body height, head length and body weight in bighead carp. The dashed and solid lines indicated the chromosome-wide and genome-wide significance thresholds.

Table 4
Associations between genotypes of SNPs and growth traits in bighead carp.

Loci	Genotypes	N	BL (cm)	BH (cm)	HL(cm)	BW(Kg)
ArTP53BP2	CC	19	30.91 ± 3.33^{a}	9.41 ± 1.02^{a}	10.30 ± 1.14^{a}	0.67 ± 0.17^{a}
c.813 T>C	TC	42	28.66 ± 3.51^{ab}	8.67 ± 1.15^{ab}	9.52 ± 1.16^{ab}	0.53 ± 0.20^{ab}
	TT	21	26.48 ± 3.56^{b}	8.02 ± 1.05^{b}	8.90 ± 1.05^{b}	0.42 ± 0.18^{b}

Significant differences at P<0.05 are labeled with different letters.

and found that proportion of the distorted markers (7.3%) was much lower than that estimated from the previous mapping family (16.0%) (Zhu et al., 2014). This marked discrepancy in the ratio of distorted markers may also be due to different mapping families used in these studies, resulting in various mortality rates of the progenies. Like tilapia (Kocher et al., 1998) and silver carp (Guo et al., 2013b), the distorted markers were clustered into segregation distortion region (SDR) on both present and previous genetic maps of bighead carp. Interestingly, four SSR markers (HysdE10982-1, Arsd90, Hysd540-2 and Hysd3112-1) on three LGs (LG4, LG15 and LG19) showed segregation distortion in both present and previous map (Zhu et al., 2014), suggesting that these markers might be linked to deleterious or lethal genes in bighead carp.

4.2. Comparative genome mapping

With the advantage of long conserved flanking sequences of microsatellite markers, a microsatellite-based high-density genetic map is a powerful tool for comparative genomic study between non-model species and model species (Guyomard et al., 2012; Woods et al., 2000). In this study, the proportion of microsatellite markers (75.3%) uniquely aligned to zebrafish genome was much higher than that of SNP markers mapped to related species (Carlson et al., 2015). Most markers (80.8%) in the present map could be located into syntenic boxs in zebrafish genome, which supply robust evidence for genomic conservation and detailed intra-chromosomal rearrangements between bighead carp and zebrafish. Furthermore, LG9 of the bighead carp map was syntenic to two chromosomes (Chr 10 and Chr 22) of zebrafish, confirming our previous findings in bighead carp (Zhu et al., 2015) and other studies in grass carp (Wang et al., 2015a; Xia et al., 2010). Our results further support the hypothesis that Chr10 and Chr22 of zebrafish are formed by the fission of an ancestral chromosome of the cyprinid fishes (Zhu et al., 2015).

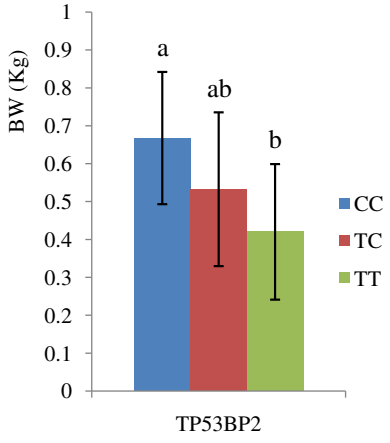

Fig. 4. Differences of observed body weight traits among *TP53BP2* genotypes in verified population. The mean and standard deviation for body weight for each genotypes were provided.

4.3. QTL for growth traits

It has been generally adopted that most economically valuable traits of domestic animals, like growth, are quantitative traits that affected by multiple genes located in different genomic regions (Goddard and Hayes, 2009). QTL mapping has been proved to be an efficient approach to identify quantitative trait-associated markers or candidate genes, and has been successfully applied in some aquaculture species (Tong and Sun, 2015; Yue, 2014), such as Atlantic salmon (Baranski et al., 2010; Moen et al., 2015), rainbow trout (Reid et al., 2005) and tilapia (Cnaani et al., 2003), Arctic charr (Kuttner et al., 2011), Asian seabass (Wang et al., 2015b) and Japanese flounder (Song et al., 2012a). In this study, three significant and 8 suggestive QTL located at three QTL intervals with a PVE range of 18.6%–25.5%, indicating that some of these QTLs may have major effects and others may have minor effects on growth in bighead carp. In addition, the parameters of four growth traits of bighead carp were found to be highly correlated with each other in this study. As expected, these traits had similar distribution patterns of QTLs which were mainly clustered into three main regions on the genetic map, indicating the pleiotropic effect of a QTL for multiple traits. This phenomenon has also been reported in several aquaculture species, such as Atlantic salmon (Baranski et al., 2010; Tsai et al., 2015), Asian seabass (Xia et al., 2013) and Zhikong Scallop (Jiao et al., 2014).

The resolution of a genetic map has a vital effect on the accuracy of QTL mapping (Voorrips, 2002). A high-density linkage map can narrow down QTL to rather small genomic regions (Wang et al., 2011a). In this study, the average of eleven QTL intervals was 1.41 cM, which was similar or smaller than those detected based on the same mapping strategies in some other aquaculture fishes (Song et al., 2012b; Wang et al., 2011a). Based on the size of bighead carp genome (0.9 Gb, Prof. Shunping He, unpublished data), the average recombination rate across all LGs was estimated to be ~1.81 cM/Mb. Thus, the average physical length of the QTLs in this study was approximately 0.78 Mb, which facilitated the identification of candidate genes for growth in bighead carp in this study.

4.4. Candidate genes

Comparative genomics with model species or closely-related species is an effective way to identify candidate genes that may harbor in QTL intervals (Wang et al., 2011a; Wang et al., 2011b). Potential candidate genes for growth traits have been detected in some aquaculture species, such as *PVALB1*, *GH*, cathepsin D, *IFABP-a* and *ACOX1* in Asian seabass (Wang et al., 2006; Wang et al., 2011a; Wang et al., 2015b; Xia et al., 2013). In this study, three putative growth-related genes were identified from QTL mapping and subsequent comparative genomics analyses, and one of the gene *TP53BP2* located in a genome-wide significant QTL on LG9 may be an possible candidate gene for growth. Interestingly, a SNP mutation *TP53BP2*-c813C>T in exon 7 showed significant associations with growth in the verification population, further indicating that *TP53BP2* may be a candidate growth gene in bighead carp. The function of the *TP53BP2* was rarely investigated in fish so far. However, a homolog of the *TP53BP2* in human was reported to have significant associations with tumor growth and play a crucial role in regulation of cell proliferation and apoptosis (Takahashi et al., 2004). Our results

demonstrate the potentials to use QTL information in breeding programs of bighead carp.

5. Conclusions

In summary, a novel genetic linkage map based on microsatellite markers was constructed for bighead carp. 905 SSRs were assigned onto 24 LGs which are equal to chromosome number of the haploid genome of the species. The consensus map spanned 1631.7 cM with a genome coverage of 94.7% and a resolution of 1.8 cM/locus. Three genome-wide and 8 group-wide significant QTL with 18.6–25.5% PVE were detected in three genomic regions. Comparative genomics analyses based on QTL-linked SSR markers and association study between SNP genotypes and growth traits indicate that *TP53BP2* may be one of the candidate genes for growth regulation in bighead carp. These results supply a basis for QTL fine mapping and identification of major growth-related genes and gene markers which would make contributions to marker-assisted selection in bighead carp.

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Competing interests

The authors declare that they have no competing interests.

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